

***Remarks***

Upon entry of the foregoing amendment, claims 1-37 are pending in the application, with claims 1, 14, 25 and 27 being the independent claims. Claims 38 and 39 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Claims 3-13, 16-24, 29-31 and 33-37 were amended only to eliminate multiple dependencies and correct spelling errors. Hence, no new matter has been added by the amendment and entry and consideration of the same is respectfully requested.

***Conclusion***

It is respectfully believed that the present application is in condition for examination. Early notice to this effect is earnestly solicited. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

  
Eric K. Steffe  
Attorney for Applicants  
Registration No. 36,688

Date: 9/20/02  
1100 New York Avenue, N.W.  
Suite 600  
Washington, D.C. 20005-3934  
(202) 371-2600

**Version with markings to show changes made**

***In the Claims:***

The pending claims 3-13, 16-24, 29-31 and 33-37 were substituted by the following claims 3-13, 16-24, 29-31 and 33-37.

3. (Once amended) Method according to claim 1 [or 2], characterized [characterised] in that the prokaryotic cell is *E. coli*.

4. (Once amended) Method according to [one of claims] claim 1 [to 3], characterized [characterised] in that the [the] following steps are carried out:

- a) the DNA encoding the tPA, tPA variant, K2S molecule or K2S variant is amplified by PCR;
- b) the PCR product is purified;
- c) said PCR product is inserted into a vector comprising the DNA coding for OmpA signal peptide and the DNA coding for gpIII in such a way that said PCR product is operably linked upstream to the DNA coding for the OmpA signal sequence and linked downstream to the DNA coding for gpIII of said vector;
- d) that a stop codon is inserted between said tPA, tPA variant, K2S molecule or K2S variant and gpIII;
- e) said vector is expressed by the prokaryotic cell;
- f) the tPA, tPA variant, K2S molecule or K2S variant is purified.

5. (Once amended) Method according to [one of claims] claim 1 [to 4],  
characterized [characterised] in that the vector is a phagemid vector comprising the DNA  
coding for OmpA signal peptide and the DNA coding for gpIII.

6. (Once amended) Method according to [one of claims] claim 1 [to 5],  
characterized [characterised] in that the vector is the pComb3HSS phagemid.

7. (Once amended) Method according to [one of claims] claim 1 [to 6],  
characterized [characterised] in that the DNA Sequence of OmpA linked upstream to  
K2S comprises the following sequence or a functional variant thereof or a variant due to  
the degenerate nucleotide code:

ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTCGCTACCG  
TGGCCCAGGCAGGCCTCTGAGGGAAACAGTGACTGCTACTTGGGAATGGGTC  
AGCCTACCGTGGCACGCACAGCCTCACCGAGTCGGTGCCCTGCCTCCCG  
TGGAAATTCCATGATCCTGATAGGCAAGGTTACACAGCACAGAACCCAGTG  
CCCAGGCACTGGGCCTGGCAAACATAATTACTGCCGAATCCTGATGGGGA  
TGCCAAGCCCTGGTGCCACGTGCTGAAGAACCGCAGGCTGACGTGGAGTA  
CTGTGATGTGCCCTCCTGCTCCACCTGCCGCTGAGACAGTACAGCCAGCCT  
CAGTTTCGCATCAAAGGAGGGCTTCGCCGACATGCCCTCCCACCCCTGGC  
AGGCTGCCATTTGCCAAGCACAGGAGGTGCCGGAGAGCGGTTCTGTG  
CGGGGGCATACTCATCAGCTCCTGCTGGATTCTCTGCCGCCACTGCTTCC

AGGAGAGGTTCCGCCCCACCACCTGACGGTGATCTTGGCAGAACATACCG  
GGTGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGAAAAATACATTGT  
CCATAAGGAATTGATGATGACACTTACGACAATGACATTGCGCTGCTGCAG  
CTGAAATCGGATTCGTCCCGCTGTGCCAGGAGAGCAGCGTGGTCCGCACTG  
TGTGCCTCCCCCGGCGACCTGCAGCTGCCGGACTGGACGGAGTGTGAGCT  
CTCCGGCTACGGCAAGCATGAGGCCTTGTCTCCTTCTATTGGAGCGGCTG  
AAGGAGGCTCATGTCAGACTGTACCCATCCAGCCGCTGCACATCACAAACATT  
TACTTAACAGAACAGTCACCGACAACATGCTGTGTGCTGGAGACACTCGGAG  
CGGCGGGCCCCAGGCAAACATTGCACGACGCCTGCCAGGGCGATTGGGAGG  
CCCCCTGGTGTCTGAACGATGGCCGCATGACTTGGTGGCATCATCAGC  
TGGGGCCTGGCTGTGGACAGAAGGATGTCCGGGTGTGTACACAAAGGTTA  
CCAACCTACCTAGACTGGATTGACAAACATGCGACCG (SEQ ID NO:2)

8. (Once amended) Method according to [one of claims] claim 1 [to 7],  
characterized [characterised] in that the DNA Sequence of OmpA comprises the  
following sequence:

ATGAAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTCGCTACCG  
TGGCCCAGGCGGCC (SEQ ID NO:3)

9. (Once amended) Method according to [one of claims] claim 1 [to 8],  
characterized [characterised] in that the DNA Sequence of OmpA consists of the  
following sequence:

ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTCGCTACCG  
TGGCCCAGGCAGGCC (SEQ ID NO:3)

10. (Once amended) Method according to [one of claims] claim 1 [to 9],  
characterized [characterised] in that the DNA of the tPA, tPA variant, K2S molecule or  
K2S variant is preceded [preceeded] by a lac promoter [promotor] and/or a ribosomal  
binding site.

11. (Once amended) Method according to [one of claims] claim 1 [to 10],  
characterized [characterised] in that the DNA coding for the tPA, tPA variant, K2S  
molecule or K2S variant is selected from the group of DNA molecules coding for at least  
90% of the amino acids 87 - 527, 174 - 527, 180 - 527 or 220 - 527 of the human tissue  
plasminogen activator protein.

12. (Once amended) Method according to [one of claims] claim 5 [to 11],  
characterized [characterised] in that the DNA Sequence of K2S comprises the following  
sequence or a functional variant thereof or a variant due to the degenerate nucleotide  
code:

TCTGAGGGAAACAGTGACTGCTACTTGGGAATGGGTAGCCTACCGTGG  
CACGCACAGCCTACCGAGTCGGTGCCTCCTGCCTCCGTGGAATTCCA  
TGATCCTGATAGGCAAGGTTACACAGCACAGAACCCAGTGCCCAGGC  
ACTGGGCCTGGCAAACATAATTACTGCCGGAATCCTGATGGGGATGCC  
AAGCCCTGGTGCCACGTGCTGAAGAACCGCAGGCTGACGTGGGAGTACT

GTGATGTGCCCTCCTGCTCCACCTGCGGCCTGAGACAGTACAGCCAGCCT  
CAGTTCGCATCAAAGGAGGGCTTCGCGACATGCCCTCCACCCCTG  
GCAGGCTGCCATCTTGCCAAGCACAGGAGGTGCCCCGGAGAGCGGTTTC  
CTGTGCGGGGGCATACTCATCAGCTCCTGCTGGATTCTCTGCCGCCA  
CTGCTTCCAGGAGAGGTTCCGCCACCACCTGACGGTGATCTGGCA  
GAACATACCGGGTGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGA  
AAAATACATTGTCCATAAGGAATTGATGATGACACTTACGACAATGACA  
TTGCGCTGCTGCAGCTGAAATCGGATTCGTCCCGCTGTGCCAGGAGAGC  
AGCGTGGTCCGCACTGTGTGCCTCCCCCGGCGGACCTGCAGCTGCCGA  
CTGGACGGAGTGTGAGCTCTCCGGCTACGGCAAGCATGAGGCCTGTCTC  
CTTCTATTGGAGCGGCTGAAGGAGGCTATGTCAGACTGTACCCATCC  
AGCCGCTGCACATCACAAACATTACTAACAGAACAGTCACCGACAACA  
TGCTGTGTGGAGACACTCGGAGCGGCGGGCCAGGCAAACATTGCA  
CGACGCCTGCCAGGGCGATTGGGAGGGCCCTGGTGTCTGAACGAT  
GGCCGCATGACTTGGTGGCATCATCAGCTGGGCCTGGCTGTGGAC  
AGAAGGATGTCCGGGTGTGTACACAAAGGTACCAACTACCTAGACTG  
GATTGACAAACATGCGACCGTGA (SEQ ID NO:4).

13. (Once amended) Method according to [one of claims] claim 5 [to 12],  
characterized [characterised] in that the DNA Sequence of K2S consists of the following  
sequence:

TCTGAGGGAAACAGTGAUTGCTACTTGGGAATGGGTAGCCTACCGTGG  
CACGCACAGCCTACCGAGTCGGTGCCTCCTGCCTCCGTGGAATTCCA

TGATCCTGATAGGCAAGGTTACACAGCACAGAACCCCCAGTGCCCAGGC  
ACTGGGCCTGGCAAACATAATTACTGCCGGAATCCTGATGGGGATGCC  
AAGCCCTGGTGCACGTGCTGAAGAACCGCAGGCTGACGTGGGAGTACT  
GTGATGTGCCCTCCTGCTCCACCTGCGGCCTGAGACAGTACAGCCAGCCT  
CAGTTCGCATCAAAGGAGGGCTTCCGCCGACATGCCCTCCCACCCCTG  
GCAGGCTGCCATCTTGCCAAGCACAGGAGGTGCCCCGGAGAGCGGTT  
CTGTGCGGGGGCATACTCATCAGCTCCTGCTGGATTCTCTGCCGCCA  
CTGCTTCCAGGAGAGGTTCCGCCACCACCTGACGGTGATCTGGGCA  
GAACATACCGGGTGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGA  
AAAATACATTGTCCATAAGGAATTGATGATGACACTACGACAATGACA  
TTGCGCTGCTGCAGCTGAAATCGGATTGCTCCGCTGTGCCAGGAGAGC  
AGCGTGGTCCGCACTGTGTGCCTCCCCGGGGACCTGCAGCTGCCGGA  
CTGGACGGAGTGTGAGCTCTCCGGCTACGGCAAGCATGAGGCCTGTCTC  
CTTCTATTGGAGCGGCTGAAGGAGGCTATGTCAGACTGTACCCATCC  
AGCCGCTGCACATCACAAACATTACTAACAGAACAGTCACCGACAACA  
TGCTGTGTGCTGGAGACACTCGGAGCGGCGGGCCCCAGGCAAACCTGCA  
CGACGCCTGCCAGGGCGATTGGGAGGCCCCCTGGTGTCTGAACGAT  
GGCCGCATGACTTGGTGGCATCATCAGCTGGGCCTGGCTGTGGAC  
AGAAGGATGTCCGGGTGTACACAAAGGTTACCAACTACCTAGACTG  
GATTCGTGACAACATGCGACCGTGA (SEQ ID NO:4).

16. (Once amended) DNA molecule according to claim 14 [or 15], characterized in that said DNA sequence consists of the following sequence:

ATGAAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTCGCTACCG  
TGGCCCAGGCAGGCCTCTGAGGGAAACAGTGACTGCTACTTGGGAATGGGTC  
AGCCTACCGTGGCACGCACAGCCTACCGAGTCGGTGCCCTCCTGCCTCCCG  
TGGAATTCCATGATCCTGATAGGCAAGGTTACACAGCACAGAACCCAGTG  
CCCAGGCAGTGGCCTGGCAAACATAATTACTGCCGGAATCCTGATGGGA  
TGCCAAGCCCTGGTGCCACGTGCTGAAGAACCGCAGGCTGACGTGGAGTA  
CTGTGATGTGCCCTCCTGCTCCACCTGCCGCTGAGACAGTACAGCCAGCCT  
CAGTTCGCATCAAAGGAGGGCTTCGCCGACATGCCCTCCCACCCCTGGC  
AGGCTGCCATTTGCCAAGCACAGGAGGTCGCCGGAGAGCGGTTCTGT  
GCGGGGGCATACTCATCAGCTCCTGCTGGATTCTCTCTGCCGCCACTGCTT  
CCAGGAGAGGTTCCGCCACCACCTGACGGTGATCTGGCAGAACATA  
CCGGGTGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGAAAAATACA  
TTGTCCATAAGGAATTGATGATGACACTTACGACAATGACATTGCGCTGC  
TGCAGCTGAAATCGGATTGTCCTGCTGTGCCAGGAGAGCAGCGTGGTCC  
GCACTGTGCCTCCCCCGGCGGACCTGACGCTGCCGACTGGACGGAGT  
GTGAGCTCTCCGGTACGGCAAGCATGAGGCCTGTCTCCTTCTATTGG  
GCGGCTGAAGGAGGCTATGTCAGACTGTACCCATCCAGCCGCTGCACATC  
ACAACATTACTAACAGAACAGTCACCGACAACATGCTGTGCTGGAGAC  
ACTCGGAGCGGCGGGCCCCAGGCAAACCTGACGACGCCGCCAGGGCGAT  
TCGGGAGGCCCCCTGGTGTCTGAACGATGCCGCATGACTTGGTGGCA  
TCATCAGCTGGGCCTGGCTGTGGACAGAAGGATGTCCGGGTGTACAC  
AAAGGTTACCAACTACCTAGACTGGATTGTCGACAACATGCGACCG (SEQ ID  
NO:5).

17. (Once amended) DNA molecule according to [one of claims] claim 14 [to 16], characterized in that said DNA sequence b) is coding for at least 90% of the amino acids 87 - 527 of the human tissue plasminogen activator protein.

18. (Once amended) DNA molecule according to [one of claims] claim 14 [to 17], characterized in that said DNA sequence b) is coding for at least 90% of the amino acids 174 - 527 of the human tissue plasminogen activator protein.

19. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 18], characterized in that said DNA sequence b) is coding for at least 90% of the amino acids 180 - 527 of the human tissue plasminogen activator protein.

20. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 19], characterized in that said DNA sequence b) is coding for at least 90% of the amino acids 220 - 527 of the human tissue plasminogen activator protein.

21. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 20], characterized in that said DNA sequence a) is hybridizing under stringent conditions to the following sequence:

ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCG  
TGGCCCAGGCGGCC (SEQ ID NO:6).

22. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 21], characterized in that said DNA sequence a) consists of the following sequence:

ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCG  
TGGCCCAGGCAGGCC (SEQ ID NO:6).

23. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 22], characterized in that said DNA sequence b) is hybridizing under stringent conditions to the following sequence:

TCTGAGGGAAACAGTGAUTGCTACTTGGGAATGGGTCAACCTACCGTGGC  
ACGCACAGCCTACCGAGTCGGGTGCCTCCTGCCTCCGTGGAATTCCATG  
ATCCTGATAGGCAAGGTTACACAGCACAGAACCCAGTGCCCAGGCAGT  
GGGCCTGGCAAACATAATTACTGCCGAATCCTGATGGGGATGCCAAGC  
CCTGGTGCCACGTGCTGAAGAACCGCAGGCTGACGTGGAGTACTGTGAT  
GTGCCCTCCTGCTCCACCTGCGGCCTGAGACAGTACAGCCAGCCTCAGTT  
CGCATCAAAGGAGGGCTTCGCCGACATGCCCTCCACCCCTGGCAGGCT  
GCCATCTTGCCAAGCACAGGAGGTGCCGGAGAGCGGTTCTGTGCGGG  
GGCATACTCATCAGCTCCTGCTGGATTCTCTGCCGCCACTGCTTCCAGG  
AGAGGTTCCGCCACCCACCTGACGGTGATCTGGCAGAACATACCGGG  
TGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGAAAAATACATTGTCC  
ATAAGGAATTGATGACACTTACGACAATGACATTGCGCTGCTGCAGCT  
GAAATCGGATTCGTCCCGCTGTGCCAGGAGAGCAGCGTGGTCCGACTGTG  
TGCCTTCCCCGGCGGACCTGCAGCTGCCGGACTGGACGGAGTGTGAGCTCT  
CCGGCTACGGCAAGCATGAGGCCTGTCTCCTTCTATTGGAGCGGCTGAA

GGAGGCTCATGTCAGACTGTACCCATCCAGCCGCTGCACATCACAAACATT  
CTAACAGAACAGTCACCGACAACATGCTGTGCTGGAGACACTCGGAGCG  
GCGGGCCCCAGGCAAACATTGCACGACGCCTGCCAGGGCGATTGGGAGGCC  
CCCTGGTGTGTCTGAACGATGCCGCATGACTTGGTGGGCATCATCAGCTG  
GGGCCTGGGCTGTGGACAGAAGGATGTCCCGGGTGTACACAAAGGTTAC  
CAACTACCTAGACTGGATTGTGACAAACATGCGACCGTGA (SEQ ID NO:7).

24. (Once amended) DNA molecule according to [any one of claims] claim 14 [to 23], characterized in that said DNA sequence b) consists of the following sequence:

TCTGAGGGAAACAGTGAUTGCTACTTGGGAATGGGTAGCCTACCGTGGC  
ACGCACAGCCTACCGAGTCGGGTGCCTCCTGCCCTCCGTGGAATTCCATG  
ATCCTGATAGGCAAGGTTACACAGCACAGAACCCCAGTGCCAGGCAGT  
GGCCTGGCAAACATAATTACTGCCGAATCCTGATGGGATGCCAAGC  
CCTGGTGCCACGTGCTGAAGAACCGCAGGCTGACGTGGAGTACTGTGAT  
GTGCCCTCCTGCTCCACCTGCCCTGAGACAGTACAGCCAGCCTCAGTT  
CGCATCAAAGGAGGGCTTCGCCGACATGCCCTCCACCCCTGGCAGGCT  
GCCATTTGCCAAGCACAGGAGGTGCCGGAGAGCGGTTCTGTGCGGG  
GGCATACTCATCAGCTCTGCTGGATTCTCTGCCGCCACTGCTCCAGG  
AGAGGTTCCGCCAACCACCTGACGGTGATCTGGCAGAACATACCGGG  
TGGTCCCTGGCGAGGAGGAGCAGAAATTGAAGTCGAAAAATACATTGTCC  
ATAAGGAATTGATGACACTTACGACAATGACATTGCGCTGCTGCAGC  
TGAAATCGGATTGTGCTGCCAGGAGAGCAGCGTGGTCCGCACTGT  
GTGCCTTCCCCGGCGGACCTGCAGCTGCCGGACTGGACGGAGTGTGAGCTC

TCCGGCTACGGCAAGCATGAGGCCTTGTCTCCTTCTATTGGAGCGGCTGA  
AGGAGGCTCATGTCAGACTGTACCCATCCAGCCGCTGCACATCACAAACATT  
ACTTAACAGAACAGTCACCGACAACATGCTGTGCTGGAGACACTCGGAGC  
GGCGGGCCCCAGGCAAACTTGCACGACGCCTGCCAGGGCGATTGGGAGGC  
CCCCCTGGTGTCTGAACGATGGCCGCATGACTTGGTGGGCATCATCAGCT  
GGGGCCTGGGCTGTGGACAGAAGGATGTCCCAGGTGTACACAAAGGTTA  
CCAACCTACCTAGACTGGATTGACAAACATGCGACCGTGA (SEQ ID NO:7).

29. (Once amended) K2S protein according to claim 28 [or 29], characterized [characterised] in that it comprises a protein characterized by the following amino acid sequence or a fragment, a functional variant, an allelic variant, a subunit, a chemical derivative or a glycosylation variant thereof:

SEGNSDCYFGNGSAYRGTHSLTESGASCLPWNSMILIGKVYTAQNPSAQALGLG  
KHNCRNPDGDAKPWCHVLKNRRLTWEYCDVPSCSTCGLRQYSQPQFRIKGGL  
FADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCFQERFPPHHLTVIL  
GRTYRVVPGEEQKFEVEKYIVHKEFDDDTYDNDIALLQLKSDSSRCAQESSVV  
RTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQHL  
LNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIISWG  
LGCGQKDVPGVYTKVTNYLDWIRDNMRP\* (SEQ ID NO:11).

30. (Once amended) K2S according to [any one of claims] claim 27 [to 30], characterized [characterised] in that it consists of a protein characterized by the following amino acid sequence:

SEGNSDCYFGNGSAYRGTHSLTESGASCLPWNSMILIGKVYTAQNPSAQALGLG  
KHNÝCRNPDGDAKPWCHVLKNRRLTWEYCDVPSCSTCGLRQYSQPQFRIKGGL  
FADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCFQERFPPHHLTVIL  
GRTYRVVPGEQQKFEVEKYIVHKEFDDDTYDNDIALLQLKSDSSRCAQESSVV  
RTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQHL  
LNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIISWG  
LGCGQKDVPGVYTKVTNYLDWIRDNMRP\* (SEQ ID NO:11).

31. (Once amended) A vector containing a DNA sequence according to [any one of claims] claim 14 [to 24].

33. (Once amended) The vector pComb3HSS containing a DNA according to [any one of claims] claim 14 [to 24], wherein the expression of the gp III protein is suppressed or inhibited by deleting the DNA molecule encoding said gp III protein or by a stop codon between the gene coding for a polypeptide containing the kringle 2 domain and the serine protease domain of tissue plasminogen activator protein and the protein III gene.

34. (Once amended) A prokaryotic host cell comprising a DNA molecule according to [any one of claims] claim 14 [to 24].

35. (Once amended) A prokaryotic host cell comprising a vector according to [any one of claims] claim 31 [to 33].

36. (Once amended) An *E. coli* host cell comprising a DNA molecule according to [any one of claims] claim 14 [to 24].

37. (Once amended) An *E. coli* host cell comprising a vector according to [any one of claims] claim 31 [to 33].

Claims 38 and 39 were canceled.